

Corticosterone Inhibits the Lipid-Mobilizing Effects of Oleoyl-Estrone in Adrenalectomized Rats

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Oleoyl-estrone (OE) is an adipose-derived signal that decreases energy intake and body lipid, maintaining energy expenditure and glycemic homeostasis. Glucocorticoids protect body lipid and the metabolic status quo. We studied the combined effects of OE and corticosterone in adrenalectomized female rats: daily OE gavages (0 or 10 nmol/g) and slow-release corticosterone pellets at four doses (0, 0.5, 1.7, and 4.8 mg/d). Intact and sham-operated controls were also included. After 8 d, body composition and plasma metabolites and hormones were measured. OE induced a massive lipid mobilization (in parallel with decreased food intake and maintained energy expenditure). Corticosterone increased fat deposition and inhibited the OE-elicited mobilization of body energy, even at the lowest dose. OE enhanced the corticosterone-induced rise in plasma triacylglycerols, and corticosterone blocked the

OE-induced decrease in leptin. High corticosterone and OE increased insulin resistance beyond the effects of corticosterone alone. The presence of corticosterone dramatically affected OE effects, reversing its decrease of body energy (lipid) content, with little or no change on food intake or energy expenditure. The maintenance of glycemia and increasing insulin in parallel to the dose of corticosterone indicate a decrease in insulin sensitivity, which is enhanced by OE. The reversal of OE effects on lipid handling, insulin resistance, can be the consequence of a corticosterone-induced OE resistance. Nevertheless, OE effects on cholesterol were largely unaffected. In conclusion, corticosterone administration effectively blocked OE effects on body lipid and energy balance as well as insulin sensitivity and glycemia. (*Endocrinology* 148: 4056–4063, 2007)

OLEOYL-ESTRONE (OE) IS an adipose tissue-derived hormonal signal that elicits the decrease of body fat stores (1). This is accomplished by decreasing white adipose tissue mass (2) through apoptosis (3) and massive loss of lipid (4), accompanied by a decrease in food intake and the maintenance of energy expenditure (2). The pharmacological dosing of OE results in the loss of fat in genetic (5, 6) or dietary (7) models of obesity, even when high-lipid diets were used (8, 9). OE also decreases insulin resistance and improves hyperlipidemia, especially by lowering the levels of cholesterol (2, 6, 9–12).

The effects of an iv infusion of OE on the energy balance of adrenalectomized rats are even more marked, causing the practical exhaustion of the animal's reserves (13). In addition, the administration of OE induces a transient rise in the levels of ACTH and corticosterone in rats (14), which are not correlated with the limited changes that OE induces on the hypothalamic nuclei content of CRH (15).

Glucocorticoids are widely considered a main factor in the development of the metabolic syndrome (16) because they induce insulin resistance (17), increase the hepatic glucose output (18), and increase blood lipids (19). Stimulation of the hypothalamus-pituitary-adrenal axis often results in white adipose tissue proliferation (20), increases in proinflammatory cytokine production (21), and a derangement of the

carbohydrate-lipid energy homeostasis maintained by insulin (22). The opposite actions of glucocorticoids and OE on energy substrate handling and metabolic control of glycemia and lipidemia and the stimulation of glucocorticoid production by OE hint at glucocorticoids playing a retentive or inhibitory role on OE action, similar to that of the glucocorticoid-induced insulin resistance (17), affecting the expression and regulation of the insulin signal transduction cascade (23) as well as indirect actions such as modulation of glucose and fatty acid availability, resulting in altered responses to glucose (24). Glucocorticoids also decrease the metabolic responses to leptin, an effect that has been described as resistance to leptin (25). In general, glucocorticoids minimize the effects of agents that tend to modify the metabolic status quo by decreasing the extent of change and recovering the modified parameters to their preset homeostatic condition (26). In this context, it can be expected that physiological glucocorticoid action will tend to thwart the deep changes that OE induces on the energy and lipid economy.

Because OE has the potential to become an antiobesity drug, the establishment of a possible OE resistance elicited by glucocorticoids is of critical importance in its pharmacological development and eventual deployment. In the present study, we analyzed the effects of different doses of corticosterone on the lipid-mobilizing effects of OE on a model of adrenalectomized rats.

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Abbreviations: HDL, High-density lipoprotein; NEFA, nonesterified fatty acid; OE, oleoyl-estrone.

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Materials and Methods

Animals and experimental setup

Female Wistar rats (Harlan-Interfauna, Sant Feliu de Codines, Spain) weighing 225–245 g were used. After acclimation to the animal house, part of the animals were adrenalectomized or sham operated under

isoflurane anesthesia (d -5) and left to recover for 5 d. Bilateral removal of the adrenals was achieved through two small dorsolateral skin incisions; the glands were pulled out by holding the periadrenal fat and then excised. Sham-operated animals were handled in the same way as adrenalectomized animals except that the adrenals were not cut and removed.

All animals had free access to pellet food (maintenance chow; Panlab, Barcelona Spain) and tap water; adrenalectomized rats had the water substituted by a saline solution (9 g/liter NaCl). The rats were kept in individual cages in a light- (12 h cycle), temperature- (21–22 C), and humidity- (74–77%) controlled and quiet environment.

In addition to sham-operated controls, a group of intact rats (*i.e.* not subjected to surgery or pellet implants) were used as control of the effects of surgery. The rats received a daily gavage (from d 0 to 8) of 0.2 ml sunflower oil, alone or containing OE (OED, Barcelona, Spain) at a dose of 10 nmol/g of body weight per day. Corticosterone dosing was achieved by implanting sc in the back the same day surgery (sham or adrenalectomy, d -5) was performed, with slow-releasing, cholesterol-free corticosterone pellets (Innovative Research of America, Sarasota, FL), which liberated the hormone continuously for 21 d. Pellets of 0 (placebo), 10, 35, and 100 mg corticosterone were used, yielding daily doses of 0, 0.5, 1.7, and 4.8 mg/d.

The groups included in the present study were: 1) intact rats implanted with placebo pellets (both controls and OE); 2) sham-operated rats implanted with placebo pellets (both controls and OE); 3) adrenalectomized rats implanted with placebo pellets (both controls and OE) (all placebo-treated rats thus receiving 0 mg of corticosterone); 4) adrenalectomized rats implanted with 10-mg pellets of corticosterone (both controls and OE) (corresponding to a daily dose of 0.5 mg corticosterone); 5) adrenalectomized rats implanted with 35-mg pellets of corticosterone (both controls and OE) (corresponding to a daily dose of 1.7 mg corticosterone); and 6) adrenalectomized rats implanted with 100-mg pellets of corticosterone (both controls and OE) (corresponding to a daily dose of 4.8 mg corticosterone). In all cases the daily weight and food consumption were recorded.

The experimental setup and procedures were approved by the Ethics Committee of the University of Barcelona. All animal handling procedures were carried out following the guidelines established by the European Union and the Spanish and Catalan governments.

Sample preparation and analytical procedures

On d 8, the rats were quietly taken out of their cages and killed by decapitation in less than 30 sec within 1–2 h after the beginning of the light cycle (0800 h). Blood was recovered, left to clot, and centrifuged to obtain serum, which was kept frozen until processed. The rats were dissected, and the stomach and intestinal contents were removed; the carcass and organs were sealed in polyethylene bags, autoclaved, and thoroughly homogenized (2). Net body weight (*i.e.* crude wet weight minus stomach and intestinal content) was used for body composition analysis and calculations.

The rat paste energy content was measured using a bomb calorimeter (C-7000; Ika, Heitersheim, Germany). The paste was also used for the estimation of lipid (27), water (differential weighing after 24 h in an oven at 90 C), and protein, through Kjeldahl nitrogen analysis (Tecator; Foss, Hillerød, Denmark) and the use of a rat-specific N to protein factor (28).

The energy content of the rats at the beginning of the experiment was estimated by applying, to their surgery-day weights, the energy density found in control (oil gavage) intact rats.

Energy intake was estimated from the food consumed, which contained a metabolizable energy of 13.3 kJ/g. Energy accrual was the difference between estimated whole-body energy content on the surgery day and the energy measured (bomb calorimeter) on d 8. Mean energy expenditure was estimated as the difference between energy intake and net energy accrual.

Blood serum was used for the measurement of glucose (kit glucose PAP CP; Horiba ABX, Montpellier, France), nonesterified fatty acids (NEFAs) kit (Wako Chemicals, Neuss, Germany), total triacylglycerols (kit 11528; Biosystems, Barcelona, Spain), total and high-density lipoprotein (HDL)-cholesterol (cholesterol reagent easy; Menarini, Firenze, Italy), insulin (SRI-13K; Linco, St. Charles, MO), leptin (RL-83K; Linco), and adiponectin (MADP-60HK; Linco). Serum corticosterone levels were measured by radioimmunoanalysis after acetone deproteinization,

(29) using a specific antibody (AB1297; Chemicon International, Temecula, CA).

Statistical analysis

Statistical comparisons between groups were established by one-, two-, or three-way ANOVAs and the *post hoc* Bonferroni test, using the programs Statgraphics plus 5.1 (Manugistics, Rockville, MD) and Prism 4 (GraphPad, San Diego, CA).

Results

Corticosterone response to treatments

The serum levels of corticosterone in the different groups are presented in Fig. 1. The levels were maximal in the sham-operated rats and also in intact rats, showing the stress associated with manipulation and killing. Adrenalectomy induced significant effects ($P = 0.0133$), whereas surgery alone and OE did not affect serum corticosterone significantly. Adrenalectomized rats showed negligible basal corticosterone concentrations that, as expected, increased with the dose of corticosterone ($P = 0.0000$). Substitutive corticosterone administration resulted in levels within the span of a circadian cycle (30). Again, OE did not affect the levels of corticosterone nor was there a significant interaction between both steroids' effects.

Body composition

Table 1 shows the body weight and composition changes of intact, sham-operated, and adrenalectomized rats after treatment with OE. Treatments did not result in statistically significant differences of final body weights between the groups. However, OE induced a significant difference in body weight change during the 8-d treatment.

Body total energy content (bomb calorimeter) was unaltered by surgery: the difference in energy of control and OE rats was similar for intact and sham-operated rats but increased by the additive effects of adrenalectomy and OE.

OE-induced body lipid content differences in intact and sham-operated rats was in the range of 5–6 g, which rose to 9 g in adrenalectomized rats. Surgery caused an increase of

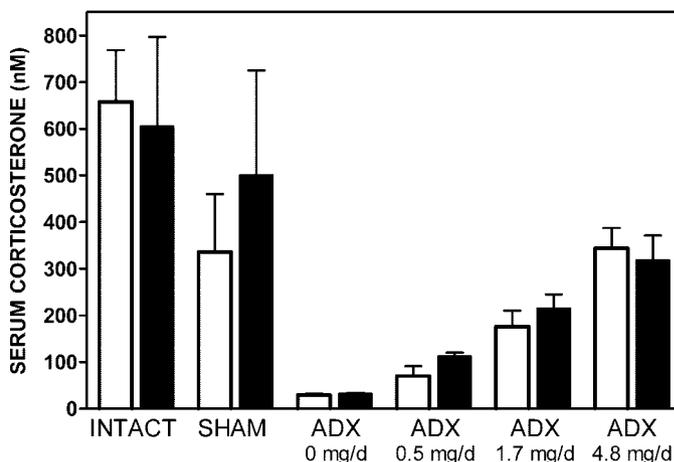


FIG. 1. Serum corticosterone of intact, sham-operated, and adrenalectomized (ADX) rats subjected to OE and corticosterone treatments. The columns represent the mean \pm SEM of six different animals. White, Controls; black, OE-treated rats.

TABLE 1. Body weight and composition changes of intact, sham-operated, and adrenalectomized rats treated 8 d with oral OE

Parameter	Units	Intact	Intact-OE	Sham	Sham-OE	Adx	Adx-OE	P			
								Surgery	Adx	OE	
Body weight	Surgery day										
	Initial			236 ± 3							
	Final		243 ± 3	234 ± 8			234 ± 3		NS	NS	NS
	Change in 8 d		245 ± 4	237 ± 4	236 ± 11	231 ± 11	235 ± 3	221 ± 4	NS	NS	NS
	Content		6.0 ± 1.5	-7.2 ± 2.3	0.6 ± 1.1	-2.8 ± 1.1	-3.8 ± 1.7	-8.4 ± 4.6	NS	NS	0.0011
Body energy (final)	Content		2.51 ± 0.12	2.34 ± 0.07	2.49 ± 0.15	2.29 ± 0.14	1.88 ± 0.05	1.53 ± 0.05	NS	0.0000	0.0058
Body lipid (final)	Density		10.4 ± 0.4	10.1 ± 0.2	11.2 ± 0.4	10.8 ± 0.3	8.3 ± 0.2	7.3 ± 0.1	0.0145	0.0000	0.0198
	Content		31.0 ± 2.0	26.3 ± 1.3	36.6 ± 3.8	30.6 ± 2.7	21.0 ± 1.5	11.8 ± 1.4	0.0282	0.0000	0.0001
Body protein (final)	Density		12.9 ± 0.8	11.4 ± 0.5	16.5 ± 1.3	14.0 ± 0.9	9.2 ± 0.6	5.6 ± 0.5	0.0003	0.0000	0.0004
	Content		43.2 ± 1.6	40.2 ± 0.6	39.1 ± 0.6	39.5 ± 1.4	41.5 ± 0.8	39.0 ± 0.9	NS	NS	NS
Body water (final)	Density		17.9 ± 0.5	17.4 ± 0.2	16.6 ± 0.6	18.1 ± 0.4	18.2 ± 0.2	18.5 ± 0.2	NS	0.0312	NS
	Content		149.2 ± 3.0	146.2 ± 2.6	138.3 ± 3.6	138.2 ± 6.8	147.9 ± 1.4	144.5 ± 2.7	0.0135	0.0334	NS
	Density		61.9 ± 0.8	63.3 ± 0.7	60.2 ± 1.6	63.4 ± 1.0	64.9 ± 0.6	68.7 ± 1.2	NS	0.0001	0.0029

The values are the mean ± SEM of six different animals. The *P* column reflects the statistical significance of the differences between groups (three-way ANOVA); NS, *P* > 0.05; Adx, Adrenalectomy.

5–6 g (sham-operated *vs.* intact controls) that turned in net loss of approximately 10 g in adrenalectomized animals.

There were no significant changes in total body protein caused by surgery, adrenalectomy, or OE treatment. Total body water, on the contrary, was not affected by OE but decreased because of surgery and increased as the consequence of loss of adrenal gland function. However, the percentage of water in the rat was unaffected by surgery and increased both with adrenalectomy and OE.

Figure 2 presents the effects of varying doses of corticosterone combined with OE on body weight, lipid, protein, and water. Corticosterone decreased body weight (*P* = 0.0033) only at the 4.8 mg/d dose; when combined with OE, the body weight *vs.* corticosterone dose curve was bell shaped, with lower values at 0 and 4.8 mg/d. OE induced significant losses of lipid (*P* = 0.0195), although this effect waned out in the presence of additional corticosterone, resulting in indistinguishable patterns for controls and OE rats already from doses as low as 0.5 mg/d. No changes attributable to OE were observed in the whole-body protein pool of intact, sham, or adrenalectomized rats, but OE decreased the protein content in rats treated with corticosterone (*P* = 0.0029). The pattern of change of total body water in rats treated with corticosterone (*P* = 0.0019) followed that described for body weight change because changes in water and lipid largely justified the modifications of body weight observed.

Table 2 presents the energy balance of intact, sham-operated, and adrenalectomized rats treated with OE. Energy intake was decreased by surgery, adrenalectomy, and OE. The latter induced decreases of 13–17% in food intake with respect to their corresponding controls. Surgery reduced food intake by 16% and adrenalectomy by almost one third. The effects of surgery, loss of adrenal gland function, and OE treatment were practically additive, with adrenalectomized and OE-treated rats eating about 60% of intact controls. Energy accrual was unaffected by surgery but decreased with adrenalectomy and OE treatment again in additive fashion. Energy expenditure decreased by surgery and adrenalectomy and was in the limit of significance for OE treatment.

The effects of corticosterone and OE on the energy balance

components of adrenalectomized rats are depicted in Fig. 3. Corticosterone induced a slow (albeit not significant) rise in energy intake, but OE-treated rats always showed lower values (*P* = 0.0062). Energy accrual in OE-treated rats increased from the marked negative values of placebo-adrenalectomized rats to lower, albeit negative, values at the highest doses (OE, *P* = 0.0000; corticosterone, *P* = 0.0017). Energy expenditure did not change significantly with increasing corticosterone.

Metabolic parameters

Plasma glucose (Table 3) was unaffected by surgery, adrenalectomy, or OE treatment. Triacylglycerols were not affected by OE but decreased in the absence of the adrenal glands because surgery alone resulted in no effects. Cholesterol behaved in a different way because here neither surgery nor the absence of adrenal glands affected its levels, which were halved by OE. The HDL-cholesterol fraction (most of the cholesterol in the rat) followed a similar pattern, but in this case adrenalectomy by itself also reduced (effect not additive with that of OE) HDL-cholesterol. Surgery increased, but adrenalectomy and OE decreased the NEFAs largely in an additive way.

Figure 4 presents the effects of corticosterone and OE on plasma metabolites of adrenalectomized rats. Under the experimental conditions tested, corticosterone dose tended to decrease glycemia (*P* = 0.0407). Adrenalectomized OE groups maintained higher glucose levels than their controls (*P* = 0.0015), irrespective of corticosterone dosing; this effect contrasts with the glucose-lowering effect of OE on intact and sham-operated rats.

Triacylglycerols were significantly increased in OE-treated rats (*P* = 0.0070), compared with those receiving corticosterone alone; the effect was not observed in placebo-treated adrenalectomized rats, which suggests its dependence on corticosterone although not dose dependent (*P* = 0.0088). The levels of NEFAs were affected by the dose of corticosterone (*P* = 0.0294) but not OE treatment.

The marked hypocholesterolemic effect of OE was maintained, even in the presence of increasing doses of cortico-

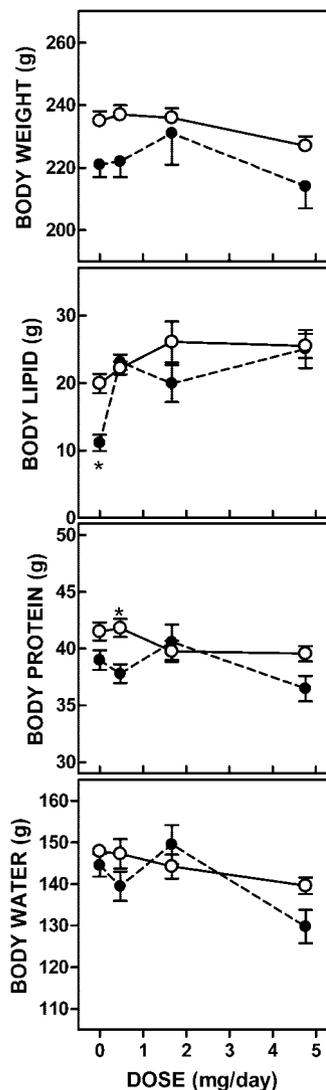


FIG. 2. Body weight and composition of adrenalectomized rats treated 8 d with oral OE receiving different doses of corticosterone. The symbols represent the mean \pm SEM of six different animals. Controls, Open circles and continuous line; OE, black circles and dashed lines. Significant differences between same-dose groups ($P < 0.05$; Bonferroni *post hoc* test) are marked with asterisks. Initial body weights of the experimental groups (mean \pm SEM) were: d -5: 236 ± 2 g; d 0: dose 0 mg/d: 234 ± 3 g; dose 0.5 mg/d: 233 ± 2 g; dose 1.7 mg/d: 234 ± 3 g; dose 4.8 mg/d: 227 ± 3 g.

sterone; at the highest doses of the latter, cholesterol levels tended to increase in parallel in both groups. The HDL-cholesterol fraction of rats receiving corticosterone increased in parallel to the dose of this hormone; however, OE treat-

ment maintained lower levels of HDL-cholesterol ($P = 0.0000$) that tended to further decrease with higher doses of corticosterone.

Insulin (Table 3) was not significantly affected by surgery, but decreased with the loss of the adrenal function and further decreased by OE treatment. Adiponectin followed roughly the same pattern, with additive effects of adrenalectomy and OE treatment. OE markedly lowered leptin levels (in additive fashion) with adrenalectomy, down to undetectable levels in placebo adrenalectomized rats.

Figure 5 presents the plasma levels of insulin, leptin, and adiponectin of adrenalectomized rats treated with OE and corticosterone. Insulin, leptin, and adiponectin increased along with corticosterone ($P = 0.0003$, $P = 0.0011$, and $P = 0.0032$, respectively). In the presence of corticosterone, the OE-induced decrease in insulin levels was reversed at the 1.7-mg/d dose. OE decreased leptin ($P = 0.0033$) and adiponectin ($P = 0.0000$). The combined treatment of OE and corticosterone resulted in a dramatic recovery of the nil leptin levels of placebo rats, up to the values of their 4.8 mg/d corticosterone controls.

Discussion

Oral OE effects on energy balance are enhanced in adrenalectomized rats, probably because of the lack of inhibition exerted by the adrenal gland-derived glucocorticoids. This is further stressed by the powerful inhibitory effect of corticosterone pellets on the lipid mobilization elicited by OE. The presence of glucocorticoids, even at a low dose emulating basal glucocorticoid production (31), results in the practical elimination of the inhibition of leptin synthesis and insulin decrease induced by OE. It also induced marked increases of serum triacylglycerols and NEFAs and, especially, a dramatic decrease of the mobilization of lipids elicited by OE.

The implantation of corticosterone pellets in adrenalectomized rats could not be fully compared with the function of normal adrenal glands because the secretion of dehydroepiandrosterone, an antiglucocorticoid (32), or those of mineralocorticoids, androgens, and estrogens as well as medullar catecholamines was not mimicked in our experimental setup. However, the pellet implant model has been widely and successfully used in studies in which a constant release of glucocorticoid was needed (31). The model was checked and found that circulating corticosterone was correlated with the pellet dose; the levels were lower than those of intact and sham-operated controls because the absence of adrenal glands prevents the spiked response to stress observed in intact and sham-operated rats. The serum corticosterone of adrenalectomized rats treated with hormone pellets agree

TABLE 2. Energy balance of intact, sham-operated, and adrenalectomized rats treated 8 d with oral OE

Parameter	Units	Intact	Intact-OE	Sham	Sham-OE	Adx	Adx-OE	P		
								Surgery	Adx	OE
Energy intake	W	2.62 ± 0.13	2.17 ± 0.08	2.20 ± 0.10	1.88 ± 0.11	1.82 ± 0.06	1.59 ± 0.12	0.0016	0.0023	0.0006
Energy accrual	W	0.10 ± 0.09	-0.04 ± 0.08	0.11 ± 0.06	-0.03 ± 0.06	-0.49 ± 0.03	-0.70 ± 0.06	NS	0.0000	0.0045
Energy expenditure (calculated)	W	2.52 ± 0.11	2.22 ± 0.14	2.05 ± 0.06	1.80 ± 0.06	2.30 ± 0.07	2.32 ± 0.11	0.0002	0.0011	0.0473

The values are the mean \pm SEM of six different animals. The P column reflects the statistical significance of the differences between groups (three-way ANOVA); NS, $P > 0.05$; Adx, adrenalectomy.

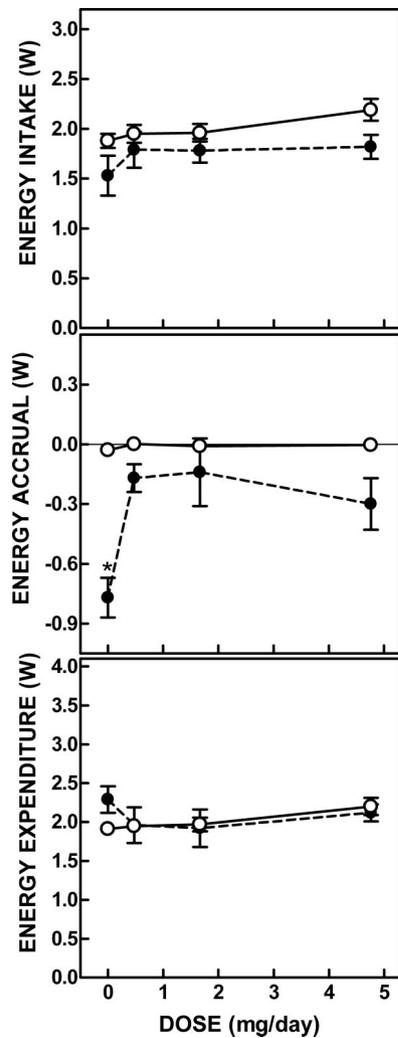


FIG. 3. Energy balance components of adrenalectomized rats treated 8 d with oral OE receiving different doses of corticosterone. The symbols represent the mean \pm SEM of six different animals. Controls, Open circles and continuous line; OE, black circles and dashed lines. The graphs show the effects on energy balance (in watts) along the whole span of the experiment (from d -5 to d 8). Significant differences between same-dose groups ($P < 0.05$; Bonferroni *post hoc* test) are marked with asterisks.

with previously described levels within the normal circadian-cycle span, with the 0.5 mg/d dose akin to the nadir and the 4.8 mg/d dose close to the maximal daily values (30, 31).

TABLE 3. Serum levels of metabolites, cytokines, and hormones of intact, sham-operated, and adrenalectomized rats treated 8 d with oral OE

Parameter	Units	Intact	Intact-OE	Sham	Sham-OE	Adx	Adx-OE	P		
								Surgery	Adx	OE
Glucose	mM	8.21 \pm 0.29	6.70 \pm 0.20	7.39 \pm 0.23	7.08 \pm 0.19	7.34 \pm 0.11	8.10 \pm 0.22	NS	NS	NS
Triacylglycerols	mM	1.62 \pm 0.2	1.47 \pm 0.3	1.71 \pm 0.1	1.58 \pm 0.1	0.67 \pm 0.06	0.51 \pm 0.07	NS	0.0000	NS
NEFAs	mM	0.40 \pm 0.03	0.36 \pm 0.03	0.53 \pm 0.04	0.40 \pm 0.03	0.33 \pm 0.05	0.21 \pm 0.01	0.0270	0.0000	0.0030
Cholesterol	mM	0.69 \pm 0.03	0.35 \pm 0.06	0.64 \pm 0.10	0.39 \pm 0.01	0.59 \pm 0.03	0.30 \pm 0.03	NS	NS	0.0000
HDL-cholesterol	mM	0.65 \pm 0.07	0.14 \pm 0.02	0.63 \pm 0.12	0.22 \pm 0.11	0.40 \pm 0.04	0.24 \pm 0.03	NS	0.0344	0.0000
Insulin	pM	292 \pm 21	210 \pm 21	204 \pm 41	157 \pm 24	161 \pm 19	100 \pm 22	NS	0.0272	0.0028
Leptin	pM	169 \pm 20	88 \pm 13	206 \pm 22	151 \pm 19	118 \pm 50	Not detectable	NS	0.0002	0.0006
Adiponectin	nM	144 \pm 12	109 \pm 1	131 \pm 7	108 \pm 18	96 \pm 16	40 \pm 3	NS	0.0004	0.0010

The values are the mean \pm SEM of six different animals. The P column reflects the statistical significance of the differences between groups (three-way ANOVA); NS, $P > 0.05$; Adx, adrenalectomy.

To minimize (or control) the variables due to surgical manipulation, we included two sets of controls in our study: controls of surgery (*i.e.* sham-operated *vs.* intact rats) and adrenalectomy (*i.e.* adrenalectomized *vs.* sham-operated), to determine the actual effects of OE on adrenalectomized animals discounting (as much as possible) the influence of surgery and stress. Surgery and adrenalectomy effects included a 13-d span (*i.e.* from d -5 to d 8), whereas OE effects included only 8 (*i.e.* from d 0 to 8). Surgery and the stress it carries affected (independently of the other treatments) body lipids and NEFAs.

Corticosterone affects body weight following a bell-shaped weight *vs.* dose curve as described (31). However, the pattern we found was less marked, probably because of a different range of doses used, the age of the rats, and the duration of the experiment (31). Body weight did not follow the lipid content changes in corticosterone-treated rats; corticosterone increased fat deposition, in agreement with previous reports (33). On the other hand, OE-induced decreases in body weight were mainly the consequence of massive losses of body lipid, with less marked changes in water and protein (2). The combination of OE and corticosterone enhanced the effects of the two treatments on body weight, with maximal losses both at low and high corticosterone doses. This pattern closely followed that of body water change. Because the proportion of body water was severalfold larger than that of lipid, the changes in lipid provoked by OE and corticosterone were translated into less marked body weight changes than the alterations in body water. The mutual influence on body water/weight by large doses of corticosterone and OE were not paralleled by changes in lipid or other parameters, suggesting that the interrelations between OE and glucocorticoids are broader than expected.

The surprising decrease of adiponectin in both adrenalectomized and OE-treated rats suggests that it may be directly or indirectly modulated by OE and glucocorticoids. The adiponectin decrease, in parallel with decreased insulin resistance (*i.e.* lower insulin and maintained glycemia), contrasts with the known parallelism of adiponectin and insulin sensitivity (34, 35) and decreased adipose mass and adiponectin secretion (36). Despite corticosterone restoring adiponectin levels, the OE-induced decrease of adiponectin (37) seems to override, in our experimental setup, the conditions favoring increased adiponectin.

The effects of oral OE on the energy levels of adrenalectomized rats are less dramatic than those observed previ-

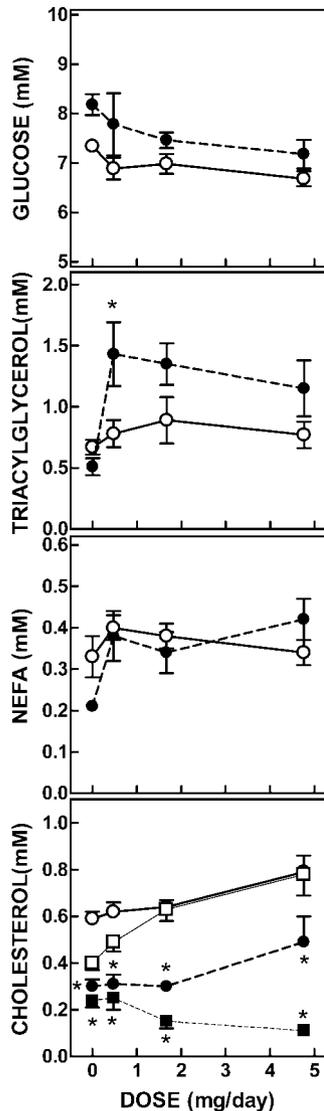


FIG. 4. Serum levels of metabolites of adrenalectomized rats treated 8 d with oral OE receiving different doses of corticosterone. The symbols represent the mean \pm SEM of six different animals. Controls, Open circles and continuous line; OE, black circles and dashed lines. In the cholesterol panel, total cholesterol: controls, open circles; OE, black circles; HDL-cholesterol: controls, open squares; OE, black squares. Significant differences between same-dose groups ($P < 0.05$; Bonferroni *post hoc* test) are marked with asterisks.

ously using iv OE (13). In the present study, we observed an additive effect of adrenalectomy and OE on body energy stores, which suggests that the mechanisms may not be coincident. Both processes affected negatively (and additively) food intake and lipid mobilization; however, OE maintained and adrenalectomy decreased energy expenditure. OE effects were centered on the active mobilization of white adipose tissue lipid and its oxidation elsewhere (38), *i.e.* maintaining the circulating levels of triacylglycerols in addition to glucose. On the other hand, the effects of adrenalectomy relied more on diminished food intake and lower energy consumption, in some way intending to protect the fat stores from exhaustion: leptin (but also insulin and adiponectin) levels decreased but not as much as in the OE-treated rats.

The marked hypocholesterolemic effects of OE (10, 12) were not paralleled in adrenalectomy alone, suggesting a fairly different handling of lipoproteins in both models.

The less marked effects of OE on sham-operated rats *vs.* intact controls may be also attributed to the higher circulating levels of glucocorticoids that this stressed group can be assumed to present (39). The presence of even low levels of corticosterone dramatically affected OE performance, mostly reversing its effects on body energy (lipid) content, with little or no change on food intake or energy expenditure. Maintenance of plasma glucose but increasing insulin levels in parallel to dose of corticosterone indicate a decrease in insulin sensitivity, an effect more marked on OE-treated rats. OE alone is known to increase the muscle ability to feed on lipids and consequently using less glucose (10). In the present setup, because the release of lipids from adipose tissue is inhibited by glucocorticoids (*i.e.* there is no significant mobilization of body lipids) glucose may be again a preferred peripheral substrate; however, OE hampers its uptake (thus maintaining high glycemia throughout) further than glucocorticoids. It can be then speculated that glucocorticoid-mediated increased hepatic glucose output (18), a critical part of its induction of insulin resistance (40), may help fulfill this relative energy deficit, but peripheral glucose uptake now requires a higher insulin secretion. Thus, paradoxically, the

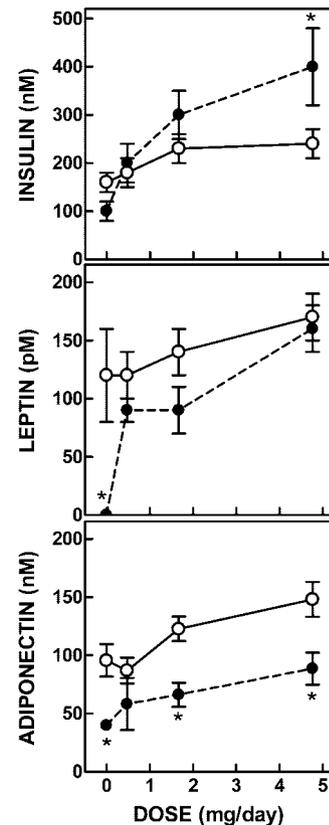


FIG. 5. Serum levels of insulin and cytokines of adrenalectomized rats treated 8 d with oral OE receiving different doses of corticosterone. The symbols represent the mean \pm SEM of six different animals. Controls, Open circles and continuous line; OE, black circles and dashed lines. Significant differences between same-dose groups ($P < 0.05$; Bonferroni *post hoc* test) are marked with asterisks.

insulin-sensitizing agent OE compounds the insulin resistance induced by glucocorticoids.

The reversal of OE effects on substrate handling, insulin resistance, and the loss of its ability to mobilize lipid can be at least in part justified by the assumption that corticosterone induces an OE resistance similar to that glucocorticoids induce on insulin (17) and leptin (25). The parallel (but more marked) breakup of the OE inhibition of leptin synthesis by corticosterone agrees with that OE resistance hypothesis. The inhibitory effect of corticosterone on OE influence on lipid mobilization (*e.g.* higher maintenance of body lipid) and disposal (*e.g.* increased triacylglycerol levels) runs parallel to the increases in adiponectin and insulin resistance induced by corticosterone on rats treated with OE.

Curiously, the effect of OE on cholesterol levels is largely unaffected by this postulated OE resistance, probably because the effect of OE on cholesterol lies more on the modulation of the cholesterol ester fraction (12) than its effect on total cholesterol; this is better seen in the different behavior of HDL-cholesterol, despite HDL being in the rat the main lipoprotein carrying this steroid (41). It may be speculated that corticosterone also affects the ability of OE to mobilize cholesterol esters, which would add to the postulated OE resistance induced by glucocorticoid.

Adrenalectomized rats receiving 0.5 mg/d dose have lower sustained circulating corticosterone levels, at the lower end of a normal daily cycle (30), than intact or sham-operated rats, even discounting the stress of handling and being killed. However, OE deeply decreases body lipids and shows its complete set of metabolic effects in animals with intact adrenals, which are blocked by the postulated OE resistance induced by corticosterone at much lower levels. This may be the consequence of other adrenal gland-secreted factors that partly counteract the OE resistance of corticosterone. The postulated secretion is not necessary for OE action because the effects of OE on adrenalectomized rats are in fact enhanced by the lack of corticosteroids. It is much more probable than that adrenal glands carry its own glucocorticoid antagonist or counteracting hormone. Further study is needed to determine the nature of this control system and its possible modulation of both OE and corticosterone function. Dehydroepiandrosterone, a known antiglucocorticoid agent (32), insulin sensitizer (42), and hormone precursor (43), production of which is limited in the rat's adrenals (33), may fulfill this role of modulator. Another possibility is the adrenal medulla-secreted catecholamines; adrenergic agents activate lipolysis (44) and enhance OE action in a synergistic way (45).

In conclusion, the effects of OE on body energy balance and its metabolic gearing to the mobilization of lipid stores are markedly controlled, and potentially stopped altogether, by natural glucocorticoids. In the rat, corticosterone administration effectively blocked most of the metabolic actions of OE. We postulate that most of these deep interactions of corticosteroids with OE metabolic effects may be the consequence of a global resistance comparable with those described for leptin and, especially, insulin. As a consequence, it is suggested that the eventual use of OE as antiobesity drug should take into consideration the evident interference of glucocorticoids (or stress) on its slimming action.

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